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Variant-specific SARS-CoV-2 shedding rates in wastewater

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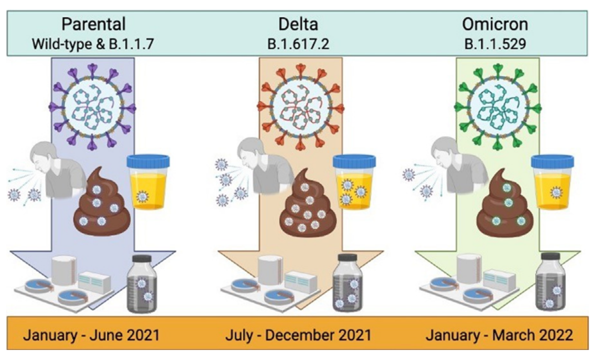
HIGHLIGHTS

- Pandemic progression and variants influence SARS-CoV-2 waste shedding rates.
- Waste shedding rates increased with Delta predominance and decreased with Omicron.
- Omicron-specific waste shedding rates ranged between 7.67 and 8.23 $\log_{10}$ gc/g-feces.
- Shedding rates were most consistent across communities during the Omicron stage.
- Waste shedding rates mirrored disease severity reported in other clinical studies.

GRAPHICAL ABSTRACT



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ABSTRACT

Previous studies show that SARS-CoV-2 waste shedding rates vary by community and are influenced by multiple factors; however, differences in shedding rates across multiple variants have yet to be evaluated. The purpose of this work is to build on previous research that evaluated waste shedding rates for early SARS-CoV-2 and the Delta variant, and update population level waste shedding rates for the more-recent Omicron variant in six communities. Mean SARS-CoV-2 waste shedding rates were found to increase with the predominance of the Delta variant and subsequently decrease with Omicron infections. Interestingly, the Delta stage had the highest mean shedding rates and was associated with the most severe disease symptoms reported in other clinical studies, while Omicron, exhibiting reduced symptoms, had the lowest mean shedding rates. Additionally, shedding rates were most consistent across communities during the Omicron stage. This is the first paper to identify waste shedding rates specific to the Omicron variant and fills a knowledge gap critical to disease prevalence modeling.

1. Introduction

Wastewater-based epidemiology (WBE) can be used to monitor public health and guide interventions to reduce health impacts (Betancourt et al., 2021; Bibby et al., 2021; Hata et al., 2021; Hillary et al., 2021). Knowledge of SARS-CoV-2 levels in sewage may provide insight into general health trends and, further, be integrated into models to estimate COVID-19 disease prevalence in a given population (Medema et al., 2020). Such models are reliant upon the waste shedding rate, or average amount of virus shed by an infected person per day, in order to generate estimations of the number of infected persons contributing to a wastewater sample (Ahmed et al., 2020; Chavarria-Miró et al., 2021; Curtis et al., 2020). Individuals infected with SARS-CoV-2 can excrete virus and/or viral RNA via feces (Cevik et al., 2021), urine (Brönimann et al., 2020; Kashi et al., 2020), saliva (Wyllie et al., 2020), and sputum

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Table 1
Predominant variants by pandemic stage.

Stage	Predominant variant(s)[d]	Dates
Parental[a]	Wild-type & B.1.1.7	January – June 2021
Delta[b]	B.1.617.2	July – December 2021
Omicron[c]	B.1.1.529	January – March 2022

[a] Parental stage was defined as the dates when wild-type (original) SARS-CoV-2 and the Alpha variant (B.1.1.7) were in circulation (refer to Section 2).

[b] Delta stage was defined as dates when ≥90 % clinical cases in the region were classified as Delta variant (B.1.617.2) infections by the Centers for Disease Control and Prevention, 2022a, 2022b, 2022c.

[c] Omicron stage was defined as dates when ≥90 % clinical cases in the region were classified as Omicron subvariant (B.1.1.529) infections by the Centers for Disease Control and Prevention, 2022a, 2022b, 2022c.

[d] Predominant variants in wastewater samples were confirmed by Translational Genomics Research Institute (TGen) (Communities A, B, and F) or GT Molecular (Community D) using tiled SARS-CoV-2 whole genome sequencing and/or variant qPCR.

(Khiabani and Amirzade-Iranaq, 2021; Li et al., 2022). Although all sources contribute to detected SARS-CoV-2 concentrations in wastewater samples, feces dominate population-level SARS-CoV-2 RNA loading in wastewater (Crank et al., 2022).

Recent findings suggest that waste shedding rates are influenced by various factors including community demographics and the progression of the pandemic, especially with respect to vaccination and variants (Prasek et al., 2022). The circulation of SARS-CoV-2 variants, in particular, may have a direct influence on waste shedding rates due to differences in disease severity, transmissibility, and/or viral loads. Yet, waste shedding rates compared across variants remain relatively unknown, especially with the rapid and constant emergence of new variants.

In this study, population-level waste shedding rates were determined for three pandemic stages, each associated with different predominant SARS-CoV-2 variants. Variant-specific waste shedding rates were then compared across stages and with respect to clinical reports on typical disease severity. Identifying and applying appropriate waste shedding rates is critical to accurately estimating COVID-19 disease prevalence through WBE modeling efforts.

2. Methods

Wastewater was monitored in six communities, four in Arizona (A, B, E, and F) and two in Florida (C and D), between January 2021 and March 2022 (Table S1). Sewage samples were collected and processed twice weekly to measure SARS-CoV-2 nucleocapsid protein 1 gene (N1) concentrations, as previously described (Betancourt et al., 2021; Prasek et al., 2022). Concentrations were subsequently adjusted for recovery efficiency rates (Table S2). Cross-laboratory experiments were performed on multiple samples to confirm the protocol could be reproduced with limited variability (Table S3, Prasek et al., 2022).

Clinical data were obtained from local and national sources (see Acknowledgements). Using criteria established by previous research, a six-day sum of reported cases was used to estimate the number of SARS-CoV-2 infected individuals who measurably contributed to a given wastewater sample (Prasek et al., 2022). Reported cases within the six-day sum were further adjusted by the CDC ascertainment ratio for COVID-19 (reported x 4.3) to approximate the total number of reported and unreported cases (Centers for Disease Control and Prevention, 2021). Waste shedding rates were calculated based on aligned wastewater and clinical data using the equation previously reported (Prasek et al., 2022; Schmitz et al., 2021).

Pandemic stages were defined by the predominant SARS-CoV-2 variant in circulation: Parental (January –June 2021), Delta (July – December 2021), and Omicron (January – March 2022) (Table 1). The Parental stage was defined by the wild-type SARS-CoV-2 and emergence of the Alpha variant (B.1.1.7), which comprised 66 % of cases by mid-April 2021 (Paul et al., 2020). During this time, other variants, including Beta (B.1.351) and Gamma (P.1), were present, but uncommon within the United States (Katella, 2022; Paul et al., 2020). Delta and Omicron stages were defined as dates when ≥90 % clinical cases in the region were classified as Delta- or Omicron-specific infections by CDC (Centers for Disease Control and Prevention, 2022a). For comparison purposes, this study considered only the time when Omicron subvariant B.1.1.529 was predominant. Variants in wastewater samples were confirmed for all communities, except Community E, via tiled SARS-CoV-2 whole genome sequencing (WGS) or qPCR by the Translational Genomics Research Institute (TGen) and GT Molecular (Figs. 1, S1 and S2).

Kruskal-Wallis H tests followed by pairwise Wilcoxon rank sum post hoc tests were performed to determine significant differences in shedding rates

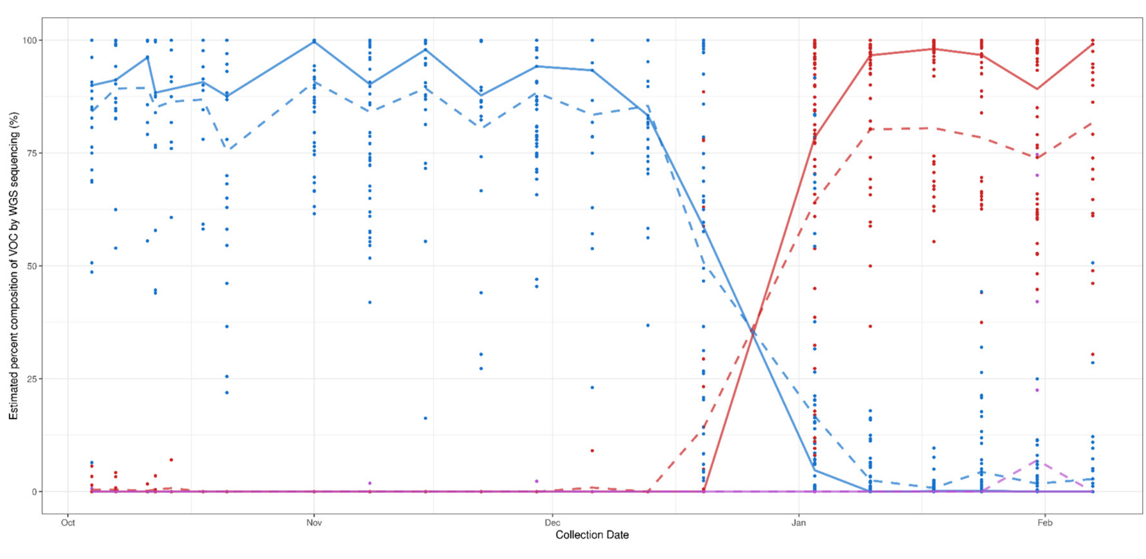

Fig. 1. SARS-CoV-2 Variants Detected in Wastewater from Communities A, B, and F
Estimated percent composition of SARS-CoV-2 variants of concern (VOC) detected in wastewater samples via tiled whole genome sequencing (WGS) in Communities A, B, and F between October 2021 – February 2022. Dashed lines indicate mean of single nucleotide polymorphisms (SNPs); solid lines indicate median of SNPs. Data processed by the Translational Genomics Research Institute (TGen).

across variant/pandemic stages (Table S4), as well as across communities (Tables S5–S7). Waste shedding calculations were performed with Microsoft Excel (version 16.47.1, 2021) and statistical comparisons were performed with R studio (R Studio Team, 2020).

3. Results

In most cases, mean waste shedding rates increased from Parental (7.57–8.45 $\log_{10}$ gc/g-feces) to Delta (8.13–9.20 $\log_{10}$), then decreased with the predominance of the Omicron variant (7.67–8.23 $\log_{10}$) (Table 2, Fig. 2). Within each community, the increase in shedding rates from Parental to Delta was statistically significant for Communities B, E, and F, but not significant in Communities A, C, and D (Table S4). In Community D, shedding rates between Parental and Delta were relatively constant. Similarly, the decrease in shedding rates from Delta to Omicron was significant in Communities C, D, and E, but not significant in Communities A, B, and F (Table S4).

Waste shedding rates were clustered closely across all communities during the Omicron stage, with mean rates falling within 0.56 $\log_{10}$ gc/g-feces of each other (Table 2 and Fig. S3). Only shedding rates between Communities A and D were significantly different; albeit, the difference was small (Table S7).

4. Discussion

4.1. Variant disease severity and shedding rates

During the transition from the Parental to the Delta stage, mean waste shedding rates in five communities increased while one remained constant. Mean rates in all six communities then decreased when Omicron emerged as the predominant variant. This shedding dynamic corroborates with clinical reports suggesting that the Delta variant resulted in more severe illness and was accompanied by higher viral loads in many patients compared to illness caused by earlier strains (Alexandar et al., 2021; Riediker et al., 2022). In contrast, Omicron-specific waste shedding rates were lower than Delta in all cases (Table 2 and Fig. 2), and infections caused by Omicron were associated with generally reduced symptoms (Mahase, 2022; Ren et al., 2022; Wolter et al., 2022; Young et al., 2022).

Table 2
Community-wide fecal shedding rates.

Community	Pandemic stage	Avg	Stdev	Med	Min	Max	n	Sig diff.[a]
A	Parental	7.72	0.57	7.49	6.27	8.37	15	P = D = O[b]
	Delta	8.13	0.60	7.77	6.26	9.21	36	
	Omicron	7.67	0.37	7.51	6.70	8.38	16	
B	Parental	7.57	0.59	7.21	6.09	8.46	49	P < D = O
	Delta	8.56	0.69	7.91	6.34	9.66	73	
	Omicron	8.08	0.49	7.88	6.79	8.77	34	
C	Parental	8.04	0.35	7.79	7.40	8.57	12	P = D > O
	Delta	8.14	0.24	8.03	7.46	8.73	78	
	Omicron	7.85	0.33	7.75	6.60	8.37	40	
D	Parental	8.26	0.20	8.17	7.82	8.75	33	P = D > O
	Delta	8.25	0.25	8.13	7.58	8.85	38	
	Omicron	7.98	0.35	7.77	7.01	8.66	20	
E	Parental	8.45	0.29	8.42	7.79	8.80	14	P < D > O
	Delta	9.20	0.52	9.09	7.63	9.68	19	
	Omicron	8.02	0.35	7.92	7.39	8.42	11	
F	Parental	8.06	0.81	7.42	5.67	9.44	78	P < D = O
	Delta	8.64	0.76	8.17	5.48	9.57	100	
	Omicron[b]	8.23	0.62	8.05	6.33	8.90	21	

All values represent SARS-CoV-2 fecal shedding rates ($\log_{10}$ gc/g-feces), except for n. Avg, average; Stdev, standard deviation; Med, median; Min, minimum; Max, maximum; n, number of samples; Sig Diff, significant difference; P, Parental; D, Delta; O, Omicron. Shedding rates differ from originally reported rates (Prasek et al., 2022) due to: 1) County-level clinical data utilized for Community D; 2) addition of samples when 6-day sum >0; and 3) expansion of Delta data set into December 2021.

[a] Refer to Tables S4–S7 for statistical results of comparisons across stages and across variants.

[b] Flow rates used during this stage were estimated based on monthly averages in 2021.

Both the Delta and Omicron variants are characterized by a lower minimal infective dose compared to wild-type SARS-CoV-2 (Riediker et al., 2022). However, the Omicron variant is also associated with lower viral loads (Sentis et al., 2022; Young et al., 2022), presented lower population-level waste shedding rates (Table 2, Fig. 2), and has a shorter duration of respiratory shedding compared to Delta (Jang et al., 2022). Additionally, Omicron has been reported to be two to three times more infectious than the Delta variant (Chen et al., 2022; Klompas and Karan, 2022). All of these factors may indicate that Omicron has a lower infective dose than Delta.

The consistency of waste shedding rates across communities in early 2022 may be due to less variability in disease expression during the Omicron stage of the pandemic. More consistent disease expression, regardless of age or population characteristics, may have resulted from Omicron's reduced severity of disease (Mahase, 2022; Ren et al., 2022; Wolter et al., 2022) buffering the potential impact of high-level contributors to viral shedding in wastewater.

4.2. Limitations for estimating waste shedding rates

Comparing population-level waste shedding over time, rates varied by variant-specific stages, and changes in shedding rates were potentially influenced by associated disease expression and variant-driven viral loads. However, the relationship between disease severity of clinical cases and waste shedding could not be directly measured in the absence of explicit patient data. Additionally, waste shedding rates were determined based on community-level analyses and should not be interpreted as representative at an individual level.

Other aspects of pandemic stages (e.g., prior infections and vaccinations) may have also contributed to shedding differences. Previous reports suggest that vaccines alleviate disease symptoms and decrease viral shedding, which influence the back-estimation of shedding rates from environmental sources (Jiang et al., 2022). During the study period, a surge in vaccination occurred in late 2021 (Centers for Disease Control and Prevention, 2022c) due to eligibility being expanded to include children ages 5 to 11 (U.S. Food and Drug Administration, 2021). This enhanced vaccination occurred just prior to, and during, the Omicron stage which may have contributed to reduced disease severity and waste shedding throughout the population during early 2022. The cumulative number of people vaccinated also increased during the Delta stage as compared to Parental (Centers for Disease Control and Prevention, 2022c), however, a similar decrease in shedding was not observed. The increased waste shedding during the Delta stage, therefore, is more likely attributable to predominant variant disease expression or other condition, though the relative influence of variables cannot be extrapolated without robust clinical data.

The ascertainment rate introduced additional uncertainty and was likely underestimated in later stages of the pandemic due to lessened symptoms and expanded home testing (Rader et al., 2021; Rubin, 2021). Notably, an underestimation of the ascertainment rate suggests that Omicron waste shedding rates, in particular, may be even lower than calculated in this study. Throughout the study, the CDC reported the ascertainment rate between 4.0 and 4.3. A sensitivity analysis indicated that ascertainment rates within this range did not result in significant differences in calculated waste shedding rates, as previously reported (Table S8; Prasek et al., 2022).

Disparities in wastewater matrices across utilities and/or laboratories were adjusted/normalized through cross-laboratory experiments on multiple samples (Table S3, Prasek et al., 2022). It is important to note, however, that uncertainties related to contributions from industrial and stormwater discharges as well as decay of SARS-CoV-2 were not considered in this study. Further research is warranted to determine the influence of such factors on waste shedding rates.

Finally, other reports have suggested different shedding rates than described here (Jones et al., 2020; Li et al., 2022; Miura et al., 2021; Pan et al., 2020; Parasa et al., 2020; Pedersen et al., 2021) which may be due to different methods of analysis, variants, shedding sources, and/or whether studies were based on clinical or wastewater data. Importantly, this study reports the combined rate from multiple waste shedding sources (e.g., feces,

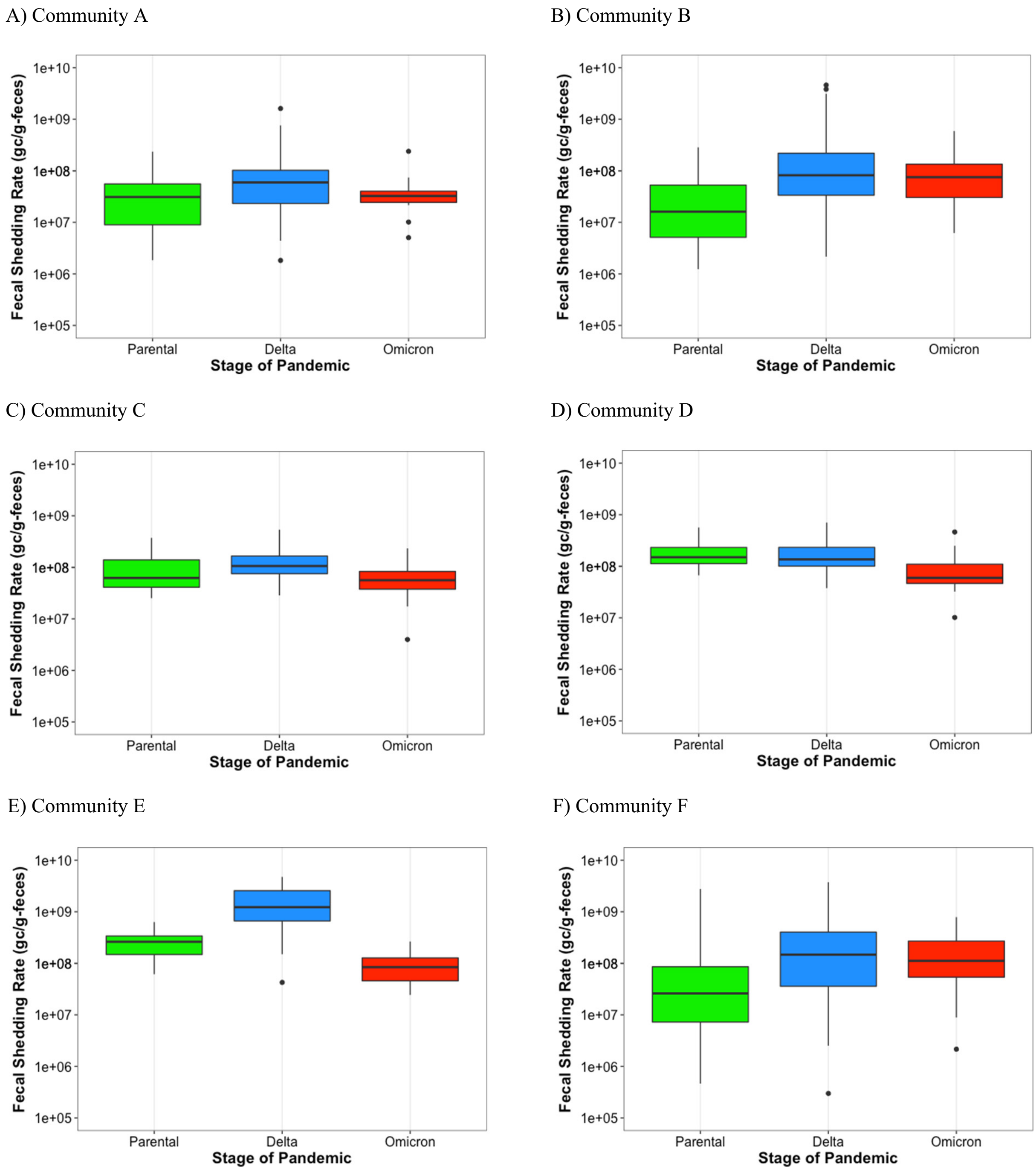

Fig. 2. Population-level SARS-CoV-2 Fecal Shedding Rates Across Pandemic Stages
Fecal shedding rates (gc/g-feces) for each community during the Parental (green), Delta (blue), and Omicron (red) stages of the pandemic. The boxes represent 50 % of the data. The horizontal line inside the box represents the mean. Whiskers represent the minimum to the lower quartile and the upper quartile to the maximum. Black dots represent outliers (>3/2 times of upper quartile or <3/2 times of lower quartile).

urine, saliva, and/or sputum) mixed in municipal wastewater, as well as focused on peak shedding (6-day sum of clinical cases). When longer intervals are utilized, case counts accumulate and calculated shedding rates decrease (Prasek et al., 2022).

4.3. Potential impacts of findings

In the absence of plentiful clinical data regarding waste shedding rates, these findings may help formulate assumptions that are critical for developing disease burden models from WBE datasets (Soller et al., 2022). This is the first study to identify differences in waste shedding rates across multiple variants, as well as identify shedding rates specific to the Omicron variant. Ultimately, waste shedding rates should be considered variant-specific and variable depending on pandemic conditions to allow for the generation of models that enhance public health preparedness and response actions.

CRediT authorship contribution statement

Sarah M. Prasek: Formal Analysis, Project Administration, Visualization, Investigation, Writing-original draft

Ian L. Pepper: Project Administration, Visualization, Investigation, Writing-original draft

Gabriel K. Innes: Visualization, Investigation, Resource, Writing – reviewing & editing

Stephanie Slinski: Methodology, Resource

Walter Q. Betancourt: Methodology, Resource

Aidan R. Foster: Resource

Hayley D. Yaglom: Resource, Data Analysis

W. Tanner Porter: Resource, Data Analysis

David M. Engelthaler: Resource, Data Analysis

Bradley W. Schmitz: Formal Analysis, Project Administration, Visualization, Supervision, Investigation, Funding acquisition, Writing – original draft.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.159165.

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